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Congenital Disorders Of Autophagy: An Emerging Novel Class Of Inborn Errors Of Neuro-Metabolism

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ABSTRACT

Single gene disorders of the autophagy pathway are an emerging, novel and diverse group of multisystem diseases in children. Clinically, these disorders prominently affect the central nervous system at various stages of development, leading to brain malformations, developmental delay, intellectual disability, epilepsy, movement disorders, and neurodegeneration, among others. Frequent early and severe involvement of the central nervous system puts the paediatric neurologist, neurogeneticist, and neurometabolic specialist at the forefront of recognizing and treating these rare conditions. On a molecular level, mutations in key autophagy genes map to different stages of this highly conserved pathway and thus lead to impairment in isolation membrane (or phagophore) and autophagosome formation, maturation, or autophagosome-lysosome fusion. Here we discuss "congenital disorders of autophagy" as an emerging subclass of inborn errors of metabolism by using the examples of six recently identified monogenic diseases: *EPG5*-related Vici syndrome, beta-propeller protein-associated neurodegeneration due to mutations in *WDR45*, *SNX14*-associated autosomal-recessive cerebellar ataxia and intellectual disability syndrome, and three forms of hereditary spastic paraplegia, SPG11, SPG15 and SPG49 caused by *SPG11*, *ZFYVE26* and *TECPR2* mutations, respectively. We also highlight associations between defective autophagy and other inborn errors of metabolism such as lysosomal storage diseases and neurodevelopmental diseases associated with the mTOR pathway, which may be included in the wider spectrum of autophagy-related diseases from a pathobiological point of view. By exploring these emerging themes in disease pathogenesis and underlying pathophysiological mechanisms, we discuss how congenital disorders of autophagy inform our understanding of the importance of this fascinating cellular pathway for central nervous system biology and disease. Finally, we review the concept of modulating autophagy as a therapeutic target and

argue that congenital disorders of autophagy provide a unique genetic perspective on the possibilities and challenges of pathway-specific drug development.

KEY WORDS: Autophagy, inborn errors of metabolism, mammalian target of rapamycin (mTOR), neurodevelopment, neurodegeneration

ABBREVIATIONS: AMPK (AMP-activated protein kinase), AR-CAID (autosomal-recessive cerebellar ataxia and intellectual disability), ATG (autophagy-related gene/protein), BPAN (beta-propeller protein-associated neurodegeneration), CLEAR (coordinated lysosomal expression and regulation), CNS (central nervous system), HSP (hereditary spastic paraplegia), iPSC (induced pluripotent stem cells), LSD (lysosomal storage disease), mTOR (mammalian target of rapamycin), mTORC1 (mammalian target of rapamycin complex 1), NBIA (neurodegeneration with brain iron accumulation), NP-C (Niemann–Pick disease, type C), SENDA (static encephalopathy of childhood with neurodegeneration in adulthood), SPG (spastic paraplegia gene), TFEB (transcription factor EB), TSC (tuberous sclerosis complex).

INTRODUCTION

Inborn errors of metabolism comprise a large group of single gene disorders that affect various aspects of cellular metabolism. Based on the specific pathway involved, such conditions can be categorized as disorders of carbohydrate metabolism, amino or organic acid metabolism, fatty acid oxidation and mitochondrial metabolism, lysosomal diseases, peroxisomal disorders, and others. While these conditions are traditionally considered “enzymopathies” due to the interruption of specific pathways with resulting accumulation of toxic or interfering intermediate products and/or reduced ability to synthesize an essential metabolite, the recent introduction of next-generation sequencing into diagnostics has resulted in the identification and characterization of novel inborn errors of metabolism.

One such cellular pathway for inborn errors of metabolism that has emerged in recent years is the autophagy pathway, with a number of genetic defects leading to inherited multisystem diseases with prominent nervous system involvement. The prominent involvement of the CNS, peripheral nerves, and skeletal muscles, often in the same patient, puts neurologists and neurogeneticists at the forefront for diagnosing and treating “congenital disorders of autophagy”. Putative single gene disorders of the autophagy pathway provide a “genetic window” into the role of autophagy in humans and will inform our understanding of this fascinating pathway and its implications for neurobiology and disease. The latter is particularly important, given that dysregulated autophagy has been implicated in many other inherited and sporadic diseases, including neurodegenerative and neurodevelopmental diseases (Pan *et al.*, 2008a, Ebrahimi-Fakhari *et al.*, 2012, Nixon, 2013, Ebrahimi-Fakhari *et al.*, 2014, Ebrahimi-Fakhari and Sahin, 2015), myopathies (Ravenscroft *et al.*, 2015), cardiovascular diseases (Lavandero *et al.*, 2013), cancer (Galluzzi *et al.*, 2015), infectious diseases (Huang and Brumell, 2014), and metabolic diseases (Christian *et al.*, 2013), all of which also affect the CNS.

We herein discuss “congenital disorders of autophagy” as an emerging subclass of inborn errors of metabolism by using the examples of six monogenic diseases of autophagy that affect the brain (*Table 1*). We also highlight associations between defective autophagy and other inborn errors of metabolism such as lysosomal storage diseases and neurodevelopmental diseases such as those associated with the mTOR pathway.

THE AUTOPHAGY PATHWAY

Autophagy (Greek for “self-eating”) is the umbrella term for essential intracellular pathways that deliver cytosolic cargo to lysosomes for degradation (reviewed in detail in Ravikumar *et al.*, 2010, Klionsky *et al.*, 2011, Kaushik and Cuervo, 2012, Feng *et al.*, 2014) (please see *Supplementary Table 1* for a glossary of autophagy-related molecules and processes). At least three subtypes of autophagy have been characterized according to the mechanism of cargo delivery: microautophagy, chaperone-mediated autophagy, and macroautophagy (*Figure 1*). *Microautophagy* employs inward invagination of the lysosomal membrane to deliver cytoplasmic cargo directly to the lysosome for degradation. *Chaperone-mediated autophagy* selectively recognizes targeting motifs (KFERQ-like motifs) on cytosolic proteins and translocates targeted proteins across the lysosomal membrane with the help of chaperone-proteins and the lysosomal adapter protein LAMP-2A. *Macroautophagy* uses the *de novo* formation of double-membrane vesicles to sequester parts of the cytosol containing proteins, lipids, and organelles. The basic principles underlying autophagy are highly conserved from yeast to mammals, highlighting the fundamental importance of this pathway for cellular homeostasis and survival. Beyond the turnover of macromolecules and organelles, recent studies have implicated a role for autophagy in related cell functions such as membrane

trafficking, regulation of energy metabolism (Efeyan *et al.*, 2015), adaptive immunity (Gomes and Dikic, 2014), and cell death (Green and Levine, 2014).

In this review, we focus on macroautophagy (hereafter referred to as autophagy), the "bulk" degradation process that is characterized by the formation of double-membrane bound autophagic vesicles that undergo a dynamic stepwise maturation process: initiation, nucleation of an isolation membrane, elongation of evolving vesicles, closure, maturation, and finally fusion with late endosomes or lysosomes (*Figure 1*). The unique double-membrane autophagic vesicle is named the autophagosome, while the fusion product of autophagosomes and lysosomes is referred to as the autolysosome. Although autophagy has been generally thought of as a non-specific degradation pathway, several specific forms of autophagy have been identified in recent years. Examples of specific forms of autophagy include the selective removal of damaged mitochondria (or "mitophagy") (Ashrafi and Schwarz, 2013), peroxisomes (or "pexophagy") (Oku and Sakai, 2010), ribosomes (or "ribophagy") (Suzuki, 2013), aberrant protein aggregates (or "aggrephagy") (Yamamoto and Simonsen, 2011), and lipid droplets (or "lipophagy") (Liu and Czaja, 2013). Posttranslational modifications such as ubiquitination or phosphorylation are critical for recruiting and tailoring the autophagy machinery to each specific cargo (Okamoto, 2014) and thus might serve as a target for developing therapeutic approaches. Ubiquitination seems to be particularly important to label cargo for autophagy, and a number of autophagy receptors with an ubiquitin-binding domain (such as p62/SQSTM1, NBR1, NDP52 or optineurin) have been identified, adding a level of plasticity and precision to the autophagy machinery (Stolz *et al.*, 2014). p62/SQSTM1 is of particular interest because it is not only an important adapter protein for ubiquitinated cargo (Wurzer *et al.*, 2015), but also a substrate for autophagic degradation (Bjorkoy *et al.*, 2005, Komatsu *et al.*, 2007a). Cells deficient

in autophagy therefore often accumulate protein aggregates that contain p62, making this a widely used assay to detect autophagy deficits (Klionsky *et al.*, 2012).

Autophagy is constitutively active to some extent in all cells at any given time in order to maintain a homeostatic balance between catabolic and anabolic processes. To dynamically adjust autophagic activity to the intracellular metabolic state and the extracellular environment, numerous signalling pathways converge to regulate autophagy initiation (*Figure 2*), many of these via mTORC1 (mammalian target of rapamycin complex 1) (Lipton and Sahin, 2014) or AMPK (AMP-activated protein kinase) (Alers *et al.*, 2012). Maintenance of baseline autophagic flux is essential to neurons, skeletal and cardiac muscle. This probably explains the increased vulnerability of these tissues to deficits in autophagy and may account for the prominent neurological and neuromuscular manifestations in primary disorders of autophagy. Specific differences in autophagy regulation and baseline autophagic flux in highly differentiated, post-mitotic cells such as neurons remain to be explored in detail, and might help to explain the preferential degeneration of specific neuronal subtypes such as cerebellar Purkinje cells or long-projecting cortical neurons that form projecting or commissural connections. On a similar note, while the role of autophagy in neuronal physiology is increasingly appreciated, the contribution of defective glial autophagy to neurodegenerative phenotypes in mice (Hara *et al.*, 2006, Komatsu *et al.*, 2006, Komatsu *et al.*, 2007b, Nishiyama *et al.*, 2007) and humans (see below) with defective autophagy remains relatively unexplored.

Following pioneering work in yeast, many proteins and mechanisms in mammalian autophagy have been uncovered. Multiple autophagy-related proteins (ATG) coordinate the different steps of autophagosome formation and turnover (*Figure 2*, please see *Supplementary Table 1* for a glossary of autophagy-related molecules and processes discussed here). The induction step in the autophagy cascade is critically determined by the phosphorylation status of the Ulk1-Atg13-

FIP200 complex, which drives the nucleation of the isolation membrane (Wong *et al.*, 2013) (*Figure 2*). Under normal nutrient conditions, active mTORC1 phosphorylates Ulk1 (unc-51-like kinase 1) and Atg13 to repress ULK1 kinase activity, thereby inhibiting autophagy (Ganley *et al.*, 2009, Hosokawa *et al.*, 2009, Jung *et al.*, 2009, Kim *et al.*, 2011). On the other hand, cell stress induced by starvation, amino acid deprivation, or growth factor withdrawal inhibits mTORC1 activity, leading to autophagy induction (Wong *et al.*, 2013).

AMPK, the second kinase at the heart of autophagy regulation, is a major positive regulator of autophagy under stress conditions. Under conditions of low intracellular energy (when the ration of AMP to ATP is high), activated AMPK induces autophagy both by phosphorylating Ulk1 (at a different site than mTORC1) and by inhibiting mTORC1 (Egan *et al.*, 2011, Kim *et al.*, 2011, Di Nardo *et al.*, 2014). Both AMPK and mTOR also control cell growth and metabolism, thereby coupling these processes to autophagy.

In the next step of the autophagic cascade, formation of an isolation membrane is initiated by a second protein complex, the Beclin1-Atg14L-Vps34 kinase complex, which enriches the initiation site with phosphatidylinositol-3-phosphate to recruit interacting regulatory proteins such as Atg9 (Funderburk *et al.*, 2010) (*Figure 2*). Atg9 serves as an important adapter molecule that recruits membranes and lipids to expand the isolation membrane (Mari *et al.*, 2010, Orsi *et al.*, 2012, Yamamoto *et al.*, 2012). On a regulatory level, phosphorylation of Beclin-1 by Akt inhibits autophagy (Wang *et al.*, 2012b), while phosphorylation at a different residue by AMPK or ULK1 promotes its integration into the Beclin1-Atg14L-Vps34 kinase complex and initiates autophagy (Kim *et al.*, 2013, Russell *et al.*, 2013). Likewise, phosphorylation of Atg9 by ULK1 is required for the efficient recruitment of additional factors to the formation site and subsequent expansion of the isolation membrane (Papinski *et al.*, 2014).

The precise subcellular location where the isolation membrane is formed remains controversial, with the endoplasmic reticulum, Golgi complex, plasma membrane, recycling endosomes, and mitochondrial membrane being putative candidates (Lamb *et al.*, 2013). It seems likely that membranes are derived from multiple sources and perhaps in a cell type- or even location-specific manner, e.g. it is conceivable that the source of autophagic membranes might be different in distal neurites compared to the cell body. Elongation of evolving autophagosomes requires two ubiquitin-like conjugation systems: the Atg5-Atg12-Atg16L1 and the LC3 (microtubule-associated protein light chain 3 or Atg8) - phosphatidylethanolamine conjugation system (Mizushima *et al.*, 2011) (*Figure 2*); the former functions as an E3 ligase that mediates the lipidation of LC3, whereas lipidated LC3 and its family members GATE16 and GABARAP are coupled to the autophagosomal membrane where they support its elongation and closure. The lipidated form of LC3, LC3-II, is of particular interest since it localizes to the inner and outer autophagosomal membrane, making this protein a widely used marker to identify and quantify autophagosome formation and turnover (Klionsky *et al.*, 2012).

Fusion with lysosomes, the final step in the lifecycle of autophagosomes, often occurs in the perinuclear region, where the bulk of lysosomes usually reside. Autophagic vesicles, however, almost certainly form everywhere in the cell and thus have to be shipped towards the perinuclear region by dynein-dependent retrograde transportation on microtubules. This process is particularly important for neurons with their long processes (Maday *et al.*, 2012, Cheng *et al.*, 2015). Local autophagic degradation, for example of damaged mitochondria (Ashrafi *et al.*, 2014), might be another important contributor to homeostasis in remote cellular regions such as distal axons, where most of the axonal autophagosomes form (Maday and Holzbaur, 2014). Lysosomal enzymes efficiently degrade the sequestered cargo, and the basic building blocks are recycled back to the cytosol for reuse. After cargo degradation,

lysosomal components are also retrieved from autolysosomes to replenish the lysosomal pool (Yu *et al.*, 2010).

Not surprisingly, lysosomal metabolism is intimately coupled to autophagy regulation. This is achieved on the transcriptional level through a recently described pathway that involves the master regulator of both lysosomal and autophagic vesicle biogenesis, the basic helix-loop-helix leucine-zipper transcription factor EB (TFEB). TFEB regulates the expression of the coordinated lysosomal expression and regulation (CLEAR) network of genes involved in lysosomal biogenesis and autophagy (Sardiello *et al.*, 2009, Palmieri *et al.*, 2011, Settembre *et al.*, 2011). Interestingly, mTORC1 associates and phosphorylates TFEB at the lysosomal membrane under conditions of nutrient sufficiency and thus prevents its translocation to the nucleus. In response to cellular energy depletion, however, mTORC1-dependent phosphorylation of TFEB is impaired, triggering the transcription of genes that encode proteins required for autophagosome formation and autophagic flux (Martina *et al.*, 2012, Roczniak-Ferguson *et al.*, 2012). Given its central role in regulating the autophagy-lysosomal pathway, TFEB has gained significant attention as a potential therapeutic target for diseases where defective autophagy and/or lysosomal dysfunction have been implicated, such as hepatic α1-antitrypsin deficiency (Pastore *et al.*, 2013), Pompe disease (Spampanato *et al.*, 2013), Parkinson's disease (Decressac *et al.*, 2013) or Huntington's disease (Tsunemi *et al.*, 2012).

CONGENITAL DISORDERS OF AUTOPHAGY

The emergence of rapid and more widely available next-generation DNA sequencing technology has led to the identification of the genetic basis of many rare diseases, including inborn errors of metabolism. Single gene disorders affecting the

autophagy pathway are increasingly identified, and genetic variants in autophagy genes are found to contribute to a number of major diseases. Here we discuss six "congenital disorders of autophagy" (*Table 1*) that predominantly affect the brain and argue for a novel subclass of inborn errors of metabolism.

VICI SYNDROME – *EPG5* MUTATIONS

First described in 1988 by Dionisi-Vici *et al.*, Vici syndrome (OMIM #242840) is a rare autosomal recessive multisystem disease with about 50 published cases to date (Byrne et al., joint submission and Dionisi Vici *et al.*, 1988, del Campo *et al.*, 1999, Chiyonobu *et al.*, 2002, Miyata *et al.*, 2007, Al-Owain *et al.*, 2010, McClelland *et al.*, 2010, Rogers *et al.*, 2011, Finocchi *et al.*, 2012, Ozkale *et al.*, 2012, Said *et al.*, 2012, Cullup *et al.*, 2013, Cullup *et al.*, 2014, Ehmke *et al.*, 2014, Filloux *et al.*, 2014, Tasdemir *et al.*, 2015). Vici syndrome is classically characterized by a set of five cardinal features that include agenesis of the corpus callosum, bilateral cataracts, hypertrophic and/or dilated cardiomyopathy, combined immunodeficiency, and skin, hair and retinal hypopigmentation (*Figure 3*). These five manifestations in addition to three features recently identified to occur in the majority of Vici patients, namely acquired microcephaly, failure to thrive and profound developmental delay, allow a clinical diagnosis that is confirmed by a positive genetic test with a specificity and sensitivity of greater than 90% (Byrne *et al.*, *joint submission*). Congenital midline defects such as a cleft lip or palate, thymic aplasia or congenital deficiency of the thymus, and hypospadia may also occur but are less frequent. Additional general findings include facial dysmorphism, dysphagia, recurrent pulmonary and mucocutaneous infections secondary to immunodeficiency, and neonatal-onset hypotonia secondary to myopathy. Chronic anaemia, renal tubular acidosis, liver dysfunction, and lung hypoplasia have also been described in a few cases (*Figure 3*).

The neurological phenotype is broad and, in addition to the prominent feature of agenesis of the corpus callosum (often with colpocephaly), which occurs in virtually all patients, involves other brain malformations such as non-lissencephalic cortical or cerebellar vermis dysplasia, pontine hypoplasia, abnormalities of the septum pellucidum, abnormal T2 signal diffusely in the thalami, and myelination defects (*Table 1* & *Figure 3*). Of these secondary features, reduced white matter bulk and under-opercularisation of the Sylvian fissures are found in the majority of patients. Post-mortem examination of a single case confirmed agenesis of the corpus callosum as well as prominent hypoplasia of the pons and cortico-spinal tracts (Byrne *et al.*, *joint submission*). Neurological sequelae are manifold and include progressive postnatal microcephaly, profound developmental delay and intellectual disability, motor impairment, nystagmus, sensorineuronal deafness, and seizures (*Table 1* & *Figure 3*). In infancy, the latter frequently evolve into epileptic encephalopathy that is often refractory to treatment. Interestingly, the reported loss of previously acquired skills as well as the occurrence of progressive microcephaly in Vici patients who survive the neonatal period, suggest a neurodegenerative component in addition to the prominent neurodevelopmental defects. This notion is corroborated by a neurodegenerative phenotype in *Epg5*-deficient *Drosophila melanogaster* (Byrne et al., *joint submission*) and transgenic mice (Zhao *et al.*, 2013a) as discussed below. The disease is usually fatal in infancy or early childhood with recurrent, severe infections and/or progressive heart failure likely being the main cause of mortality.

Using whole-exome sequencing in affected individuals, Cullup *et al.* identified recessive mutations in the *EPG5* gene on chromosome 18q12.3 as the genetic cause of Vici syndrome (Cullup *et al.*, 2013). *EPG5* consists of 44 exons encoding a protein of 2579 amino acids at maximum. The mutations identified so far map to almost the entire gene with no clear mutational hotspot (Byrne et al., *joint*

submission). Most mutations are predicted to lead to a truncated protein product, thus favouring a loss-of-function mechanism. *EPG5* is the human homolog of the metazoan-specific autophagy gene *Epg5* (ectopic P-granule autophagy protein 5), which encodes the key regulatory autophagy protein Epg5. Like many autophagy-related genes, *Epg5* first emerged in a *Caenorhabditis elegans* based genetic screening for modifiers of autophagic substrate degradation. Mutant *Epg5* in *C. elegans* as well as a gene knockdown in mammalian cells was found to result in the accumulation of dysfunctional non-degenerative autolysosomes, arguing that Epg5 is critically involved in the late stages of the autophagy cascade such as autophagosome-lysosome fusion or proteolysis within autolysosomes (Tian *et al.*, 2010, Zhao *et al.*, 2013a).

Further delineating the role of Epg5, characterization of autophagy in skeletal muscle tissue and fibroblasts from Vici patients revealed an accumulation of LC3-positive autophagic vesicles, and of the autophagy linker-proteins NBR1 and p62. This suggests a block in autophagic flux, a finding further corroborated by the accumulation of K63-polyubiquitinated proteins, which are usually targeted for autophagic degradation. Evidence for a block at the late stages of autophagy, namely during autophagosome-lysosome fusion, was provided by showing a reduction in the co-localization of both organelles (Cullup *et al.*, 2013). In retrospect, these findings are consistent with earlier studies in available muscle biopsies from Vici patients that found abundant vacuoles and dense bodies suggesting a lysosomal origin (Al-Owain *et al.*, 2010, McClelland *et al.*, 2010, Cullup *et al.*, 2013).

In summary, Epg5 deficiency in Vici syndrome leads to a critical impairment of the late stages of the autophagy pathway (*Table 1 & Figure 2*). Global reduction in autophagic flux leads to impaired development and function in a multitude of tissues and thus to a multisystem disease (*Figure 3*). Recurrent infections are a significant source of morbidity and mortality in Vici syndrome. This might be secondary to the

fact that defective autophagy impairs immunity and the clearance of intracellular pathogens in particular, as this has been recently found to critically depend on intact autophagy (Jo *et al.*, 2013). Despite the convincing role for *EPG5* mutations in human cases, *Epg5* knockout mice do not fully recapitulate the phenotype found in Vici syndrome and interestingly resemble instead key neuropathological features of the neurodegenerative disease amyotrophic lateral sclerosis (Zhao *et al.*, 2013a). Neurodegeneration mainly occurs in cortical pyramidal neurons and motor neurons of the spinal cord and is accompanied by accumulation of p62-positive and ubiquitinated protein aggregates. Not surprisingly, knockout mice display progressive motor deficits and die around 12 months of age. Muscle atrophy, fibrillations and sharp waves on electromyography indicate active denervation. Interestingly, in addition to the degenerative changes secondary to denervation, glycogen accumulation is found in skeletal muscle of *Epg5* deficient mice arguing for a "storage disease phenotype" similar to glycogen accumulation observed in muscle and other tissues from Vici syndrome patients (Al-Owain *et al.*, 2010, McClelland *et al.*, 2010, Cullup *et al.*, 2013). Although no overt developmental brain malformations were discovered, *Epg5* deficient mice were consistently found to have a reduced thickness of the corpus callosum with a partial or complete agenesis in a few knockout animals (Zhao *et al.*, 2013b). Thus, while *Epg5* knockout mice show some phenotypic similarities to Vici syndrome patients including corpus callosum dysgenesis and myopathy, core clinical features, however, are not recapitulated. Nevertheless, Vici syndrome, a multisystem disease in humans, is an important example for the concept of "congenital disorders of autophagy" as it highlights the consequences of dysfunctional autophagy for organ development and the complex role of autophagy in CNS development.

BETA-PROPELLER PROTEIN-ASSOCIATED NEURODEGENERATION (BPAN) –

WDR45 MUTATIONS

Neurodegeneration with brain iron accumulation (NBIA) is a clinically and genetically heterogeneous group of single gene disorders characterized by dysregulation of iron metabolism leading to iron deposits in the basal ganglia, particularly in the globus pallidus and substantia nigra (Horvath, 2013, Meyer *et al.*, 2015). A recently identified subtype within this group is beta-propeller protein-associated neurodegeneration (BPAN), a disease that had been previously summarized under the term Static Encephalopathy of Childhood with Neurodegeneration in Adulthood (SENDA) syndrome, based on its distinct pattern of clinical and MRI findings and its characteristic natural history (*Figure 4*). BPAN accounts for approximately 1-2 % of all NBIA cases and is the only form of the disease with an X-linked dominant inheritance pattern, although thus far only patients carrying *de novo* mutations have been reported (Haack *et al.*, 2012, Saitsu *et al.*, 2013, Nishioka *et al.*, 2015).

Clinically, BPAN is a biphasic disease that begins with global developmental delay in infancy or early childhood (*Table 1 & Figure 4*) (Hayflick *et al.*, 2013, Nishioka *et al.*, 2015). Seizures of various types including generalized tonic-clonic but also focal, absence, atonic or myoclonic seizures are frequent initial presentations, often in the context of a febrile illness. Spastic paraparesis is another commonly reported early finding. Symptoms remain relatively static until the second phase of the disease suddenly begins in adolescence or early adulthood with progressive cognitive decline, dementia, dystonia, and Parkinsonism dominating the clinical picture. The latter mainly consists of bradykinesia, rigidity and postural instability while tremor is not commonly seen. In a number of cases, these symptoms have responded to levodopa (Hayflick *et al.*, 2013). Seizures, too, usually progress in the adult phase of the disease. Sleep abnormalities, neuropsychiatric symptoms within the autistic and affective spectrum, eye movement abnormalities,

optic nerve atrophy, sensorineural hearing loss and Rett-like hand stereotypies have also been reported in a subset of patients (Hayflick *et al.*, 2013, Ohba *et al.*, 2014, Rathore *et al.*, 2014, Verhoeven *et al.*, 2014). Distinctive MRI changes allow a diagnosis in many cases (Kruer *et al.*, 2012): Iron accumulation is detectable on regular T2 sequences in the substantia nigra even at an early disease stage, while detecting iron deposits in the globus pallidum requires other techniques such as gradient-echo or T2*-weighted sequences. Hyperintensity on T1-weighted axial images with a central band of hypointense signal has been reported as a pathognomonic feature of BPAN (Kruer *et al.*, 2012, Ichinose *et al.*, 2014, Ozawa *et al.*, 2014). Cerebral atrophy, or less commonly cerebellar atrophy, and basal ganglia calcification (Van Goethem *et al.*, 2014) can also be seen. Post-mortem examination of two adult cases of BPAN revealed extensive and widespread accumulation of hyperphosphorylated tau protein in the form of neurofibrillary tangles, arguing that BPAN shares neuropathological features with classic degenerative tauopathies such as Alzheimer's disease (Hayflick *et al.*, 2013, Paudel *et al.*, 2015). BPAN is inevitably fatal with current therapeutic strategies being targeted at symptomatic relief of Parkinsonian features, dystonia, and seizures.

Using exome sequencing, two independent groups recently uncovered mutations in the *WDR45* gene as the genetic cause of BPAN (Haack *et al.*, 2012, Saitsu *et al.*, 2013). *WDR45*, also known as *WIPI4*, is located on the X-chromosome and is one of the four mammalian homologs of the core autophagy gene *Epg6* in *C. elegans* (Proikas-Cezanne *et al.*, 2004, Lu *et al.*, 2011). *WDR45* encodes a WD repeat protein, a superfamily of proteins that is characterized by repeating units with a conserved core of approximately 40 amino acids that terminate with tryptophan-aspartic acid (WD) residues. WD40 proteins have a highly symmetrical beta-propeller tertiary structure that enables them to regulate the assembly of multiprotein complexes by providing a stable anchoring platform for simultaneous

and reversible protein-protein interactions (Stirnimann *et al.*, 2010). Based on these properties, WD-repeat proteins are key components of many essential biological functions and pathways, including autophagy, signal transduction pathways, transcriptional regulation, cell cycle control, apoptosis, and vesicular trafficking (Stirnimann *et al.*, 2010).

WDR45, the WD-repeat protein mutated in BPAN, interacts with autophagy proteins Atg2 and Atg9 to regulate key steps during autophagosome formation and elongation (Behrends *et al.*, 2010, Lu *et al.*, 2011). Hence, depletion of WDR45 in mammalian cells leads to the accumulation of autophagosomes and early autophagic vesicles (Behrends *et al.*, 2010, Lu *et al.*, 2011). Investigating the effect of *WDR45* mutations in lymphoblast cell lines derived from BPAN patients, Saitsu *et al.* found that levels of WDR45 protein were strongly diminished, suggesting that the mutant protein is structurally unstable and undergoes rapid degradation (Saitsu *et al.*, 2013). Subsequently, autophagic flux is impaired, leading to an accumulation of abnormal and immature autophagic vacuoles. The latter was confirmed by showing that accumulating abnormal autophagosome precursors stain for both Atg9A and LC3, indicating improper autophagosome formation, since, under normal conditions, Atg9A only transiently associates with the autophagosome formation site and is absent from mature LC3-positive autophagosomes (Orsi *et al.*, 2012). In summary, findings in cells from BPAN patients indicate that autophagy is perturbed at an early stage (*Table 1 & Figure 2*), suggesting that autophagy dysfunction might account for the neurological sequelae of the disease. Interestingly, although BPAN is clinically thought to primarily affect the brain, WDR45 is expressed in many human tissues with the highest expression found in skeletal muscle (Proikas-Cezanne *et al.*, 2004). A muscle phenotype, however, remains to be formally investigated. The selective degeneration of neuronal populations may be explained by cell-type specific differences in autophagy and tolerance to changes in this

pathway. Neurons as non-dividing, highly differentiated cells heavily rely on intracellular degradation pathways to maintain homeostasis and normal structure and function. This is exemplified by brain-specific knockout mice of core autophagy genes showing a striking neurodegenerative phenotype similar to that found in aging-related neurodegenerative diseases (Hara *et al.*, 2006, Komatsu *et al.*, 2006). Since mice globally deficient in autophagy die shortly after birth (Kuma *et al.*, 2004, Komatsu *et al.*, 2005), *WDR45* mutations in affected human males might be inevitably lethal at an early stage. Recently reported conditional CNS-specific *WDR45* knockout mice (Nes-WDR45$^{fl/Y}$) show axonal pathology with swollen axons and accumulation of autophagy substrates p62 and ubiquitin. Although neither neurodegeneration nor iron deposition are prominent phenotypes, Nes-WDR45$^{fl/Y}$ mice, at the behavioural level, exhibit subtle motor coordination deficits and poor learning and memory, suggesting deficits in neuronal circuit formation or neurotransmission (Zhao *et al.*, 2015). Another interesting lead into the pathogenesis of BPAN comes from the recent appreciation of the role of autophagy in iron metabolism. The bioavailability of intracellular iron is critically controlled through the delivery of ferritin to autophagosomes and lysosomes for degradation (ferritinophagy), allowing release of iron into the cytoplasm (Kidane *et al.*, 2006, Asano *et al.*, 2011, Mancias *et al.*, 2014). Hence, deficits in ferritinophagy could disturb iron homeostasis, potentially contributing to the iron storage phenotype seen in NBIA that seems to confer selectivity for vulnerable brain regions.

In summary, BPAN, the second discussed congenital disorder of autophagy, is of particular importance as this disease, for the first time, confirms that genetic deficits in the autophagy pathway are indeed associated with early-onset neurodegeneration in humans (Saitsu *et al.*, 2013). This further supports a role for autophagy in the pathogenesis of an expanding list of sporadic aging-related neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease,

Huntington's disease, and others (Ebrahimi-Fakhari *et al.*, 2012, Nixon, 2013) and may provide novel insights into the role of autophagy in iron metabolism.

SNX14-ASSOCIATED AUTOSOMAL-RECESSIVE CEREBELLAR ATAXIA AND INTELLECTUAL DISABILITY SYNDROME

The childhood-onset autosomal-recessive cerebellar ataxias are a group of heterogeneous inherited diseases characterized by progressive cerebellar atrophy and prominent Purkinje cell degeneration. Next-generation sequencing has allowed a genetic and molecular resolution of a number of entities and holds great promise to facilitate an early diagnosis in many cases (Nemeth *et al.*, 2013, Pyle *et al.*, 2015). Recently, two independent groups identified truncating loss-of-function mutations in the sorting nexin gene, *SNX14*, as the cause of a clinically distinct autosomal-recessive cerebellar ataxia syndrome (Thomas *et al.*, 2014, Akizu *et al.*, 2015). Patients with bi-allelic *SNX14* mutations present with globally delayed development, hypotonia, absent speech, progressive cerebellar atrophy and ataxia, seizures, and a storage disease phenotype consisting of coarse facial features, macroglossia, hypertrychosis, kyphoscoliosis, sensorineural hearing loss, and hepatosplenomegaly in a few cases (*Table 1*) (Sousa *et al.*, 2014, Thomas *et al.*, 2014, Akizu *et al.*, 2015). On clinical grounds, these manifestations suggest overlapping disease mechanisms with lysosomal storage diseases with prominent involvement of the cerebellum such as Niemann-Pick disease type C (NP-C) or Tay-Sachs disease. Indeed, mutant *SNX14* patient fibroblasts contain enlarged lysosomes (Thomas *et al.*, 2014, Akizu *et al.*, 2015), providing a rationale to further investigate the role of *SNX14* in lysosomal turnover.

SNX14 encodes a protein of the sorting nexin family that contains two putative transmembrane domains (Mas *et al.*, 2014). Through the RGS (regulator of

G protein signalling) domain, SNX14 attenuates Gαs-coupled G protein-coupled receptor signalling. Through its PX (phox homology) domain SNX14 binds membrane phosphatidylinositol residues and is involved in intracellular trafficking. Phospholipids on membranes of different organelles provide a platform for recruiting factors that mediate membrane turnover and fusion. With regards to the autophagy pathway, this is important for key membrane fusion events such as the fusion of phosphatidylinositol 3-phosphate coated autophagosomes with phosphatidylinositol (3,5)-bisphosphate carrying lysosomes (Dall'Armi *et al.*, 2013). The importance of phospholipids for autophagy is exemplified by recent studies that show key regulatory functions in the regulation of canonical (Burman and Ktistakis, 2010) and non-canonical autophagy (Vicinanza *et al.*, 2015). In addition, cardiolipins, a subtype of phospholipids localized to the inner mitochondrial membrane, serve as a crucial signal for autophagic degradation when exposed on the surface of depolarized mitochondria (Chu *et al.*, 2013). Binding to their respective phospholipid signature, SNX14 co-localizes with the lysosome and is enriched in autophagosome containing cell fractions (Akizu *et al.*, 2015). In induced pluripotent stem cell (iPSC)-derived neuronal cells from patients with *SNX14* mutations, lysosomes are increased in size and autophagosome clearance is impaired (Akizu *et al.*, 2015). This pattern closely resembles findings in certain lysosomal storage diseases (Ebrahimi-Fakhari *et al.*, 2014). Knockdown of *SNX14* in a zebrafish model confirmed these observations and linked them to progressive Purkinje cell loss, suggesting neuronal cell death secondary to impaired autophagy (Akizu *et al.*, 2015). Purkinje cells are exquisitely sensitive to autophagy impairment and perturbations of lysosomal metabolism (Hara *et al.*, 2006, Komatsu *et al.*, 2006), which may be attributable to their size, high metabolic activity, and complex dendritic architecture. Not surprisingly, post-mortem neuropathological assessment showed a near complete absence of Purkinje cells in an affected individual with bi-allelic *SNX14* mutations (Akizu et al.,

2015).

SNX14-associated autosomal-recessive cerebellar ataxia is thus a further disease that links autophagy to neurodegeneration and highlights the importance of autophagy for Purkinje cell function and survival. Given the emerging links between cerebellar circuit dysfunction and syndromic forms of autism-spectrum disorder (Tsai *et al.*, 2012), it is interesting to note that the majority of reported patients with *SNX14* mutations show autistic-like behaviour (Akizu *et al.*, 2015).

HEREDITARY SPASTIC PARAPLEGIA - SPG11 (*SPG11*), SPG15 (*ZFYVE26*) AND SPG49 (*TECPR2*)

Hereditary spastic paraplegia (HSP) is a clinically and genetically heterogeneous group of neurodegenerative diseases that occurs at a prevalence of about 3-10 cases per 100.000 individuals (Salinas *et al.*, 2008, Blackstone, 2012). All forms of HSP share the unifying feature of distal axonal degeneration in the corticospinal tracts (Lo Giudice *et al.*, 2014). In some cases, ascending spinal tracts such as the gracile fasciculus and the spinocerebellar tracts may also be affected. Clinically, degeneration of the corticospinal tracts leads to progressive weakness and spasticity of the lower limbs. Additional manifestations in the various forms of HSP described to date range from congenital brain abnormalities such as agenesis of the corpus callosum or cerebellar dysplasia, to signs of neuronal dysfunction and neurodegeneration such as cognitive impairment, ataxia, optic nerve atrophy, epilepsy, and peripheral neuropathy (Lo Giudice *et al.*, 2014). Given the unifying feature of axonal degeneration of the long tracts, the HSPs serve as a genetically tractable model for identifying pathways that ensure axonal function and survival. Covering all modes of inheritance, more than 70 disease loci and 50 spastic paraplegia genes have been identified to date. These genes map to multiple cellular pathways and processes, including endoplasmic reticulum function, vesicle

formation, membrane trafficking, mitochondrial function, lipid metabolism, myelination, axonal transport and autophagy (Blackstone, 2012, Lo Giudice *et al.*, 2014, Noreau *et al.*, 2014). Here, we discuss SPG11, SPG15 and SPG49, three autosomal recessive forms of HSP characterized by autophagy dysfunction.

SPG11 (OMIM #604360) and SPG15 (OMIM #270700) are the most prevalent forms of autosomal recessive HSP with thin corpus callosum (Goizet *et al.*, 2009, Schule *et al.*, 2009, Pensato *et al.*, 2014). The clinical phenotype in SPG11 and SPG15 is often indistinguishable (*Table 1*) and an accurate diagnosis therefore requires genetic testing (Schule *et al.*, 2009). Patients with SPG11 or SPG15 often present with Kjellin syndrome, in which early-onset spastic paraplegia is accompanied by intellectual disability, pigmentary retinopathy, cerebellar dysfunction, and distal amyotrophy in the first or second decade of life (Kjellin, 1959). Parkinsonism is also sometimes observed.

Following linkage to the SPG11 locus on chromosome 15q (Martinez Murillo *et al.*, 1999, Shibasaki *et al.*, 2000), the gene for SPG11, *SPG11*, and its protein product spatacsin were identified (Stevanin *et al.*, 2007, Stevanin *et al.*, 2008).

For SPG15, Hughes *et al.* were first to identify the causative gene locus on chromosome 14q from two Irish families with autosomal-recessive Kjellin syndrome (Hughes *et al.*, 2001) before Hanein *et al.* pinpointed the causal gene to *ZFYVE26*, which encodes the zinc-finger protein spastizin (Hanein *et al.*, 2008). Over 100 mutations in *SPG11* and more than 20 mutations in *ZFYVE26* have been identified in patients, the majority of which are truncating, nonsense mutations (Goizet *et al.*, 2009, Pensato *et al.*, 2014).

Spastizin is a 285kDa protein comprising of a zinc finger domain, a FYVE domain, and a leucine zipper domain (Hanein *et al.*, 2008). FYVE-domain containing proteins bind phosphatidylinositol 3-phosphate, suggesting a likely function of spastizin in membrane trafficking (Kutateladze and Overduin, 2001). Spastizin is

expressed most abundantly in motor cortex, hippocampus, cerebellum, pons and spinal cord, particularly during development (Hanein *et al.*, 2008, Murmu *et al.*, 2011, Khundadze *et al.*, 2013). Intracellularly, spastizin has been observed to co-localize with a variety of structures and organelles, including endosomes and the endoplasmic reticulum and, to a likely lesser extent, microtubules, mitochondria, and the nucleus (Hanein *et al.*, 2008, Murmu *et al.*, 2011, Khundadze *et al.*, 2013, Vantaggiato *et al.*, 2013).

Zfyve26 knockout mice develop normally but by 12 months of age acquire a spastic and ataxic gait disorder accompanied by neuron loss in the motor cortex and the cerebellum, thus recapitulating the clinical phenotype of HSP patients (Khundadze *et al.*, 2013). Zebrafish models generated by morpholino-mediated knockdown of *SPG15* also show motor neuron growth impairment and morphological abnormalities (Martin *et al.*, 2012). Interestingly, knockdown of *SPG11* results in a strikingly similar phenotype (Martin *et al.*, 2012), providing genetic evidence that spastizin and spatacsin might act as part of the same pathway as previously suggested by physical interaction of the two proteins (Slabicki *et al.*, 2010) and overlapping clinical features in SPG15 and SPG11 patients (Boukhris *et al.*, 2008, Schule *et al.*, 2009).

At the cellular level, high-density Lamp1-positive membrane-bound vesicles and lipopigment accumulate in neurons of *Zfyve26* knockout mice and precede neurodegenerative changes (Khundadze *et al.*, 2013). Enlarged lysosomes are readily visible in fibroblasts derived from SPG15 patients (Renvoise *et al.*, 2014), similarly suggesting deficits in the autophagy-lysosomal and/or endosomal-lysosomal pathway. Vantaggiato *et al.* recently provided an important lead by identifying an interaction between spastizin and the Beclin 1-UVRAG-Rubicon complex of the autophagy pathway (Vantaggiato *et al.*, 2013) (*Figure 2*). As discussed above, Beclin 1 has a multifaceted role in autophagy: in association with

Atg14L and Ambra1, the Beclin 1-Vps34-Vps15 class III phosphatidylinositol 3-kinase complex is involved in autophagosome formation by regulating phosphatidylinositol 3-phosphate synthesis; separately, when interacting with cofactors UVRAG and Bif-1, Beclin 1-Vps34-Vps15 induces autophagosome maturation and endosome fusion. Finally, Rubicon, in complex with UVRAG and Beclin 1-Vps34-Vps15, functions as a negative regulator of autophagy (Liang *et al.*, 2006, Itakura *et al.*, 2008, Matsunaga *et al.*, 2009, Zhong *et al.*, 2009, Kang *et al.*, 2011). Spastizin interacts with the Beclin 1-UVRAG-Rubicon multiprotein complex and is thus involved in autophagosome maturation (Vantaggiato *et al.*, 2013, Vantaggiato *et al.*, 2014). Mutations in spastizin disrupt its interaction with Beclin 1 and inhibit autophagosome maturation, leading to an accumulation of immature autophagosomes in fibroblasts and lymphoblasts from SPG15 patients (Vantaggiato *et al.*, 2013). RNA-interference-mediated knockdown of spastizin in cultured neurons was shown to yield similar results. In addition, reduced colocalization of LC3 and Lamp1 was noted in basal and autophagy-promoting conditions, indicating a block in autophagosome-lysosome fusion (Vantaggiato *et al.*, 2013). Recently, pathogenic alterations of autophagy and lysosomal function in SPG15 have been further confirmed by showing that spastizin and the SPG11 protein spatacsin are instrumental for the reformation of autophagic lysosomes, a recycling pathway that generates new lysosomes (Chang *et al.*, 2014, Varga *et al.*, 2015). This mechanism of lysosome biogenesis maintains a pool of lysosomes that are competent to fuse with autophagosomes in order to create new autolysosomes (Yu *et al.*, 2010). Spastizin forms a complex with spatacsin and is targeted to lysosomes through spastizin's FYVE domain. Loss of either spastizin or spatacsin results in accumulation of enlarged autolysosomes, which is reversible by reintroducing wild-type spastizin. Similarly, accumulation of autophagosomes and depletion of free lysosomes is seen, suggesting a block at the autophagic lysosome reformation

stage (Chang *et al.*, 2014, Varga *et al.*, 2015). Taken together, disruption of the critical steps of autophagosome maturation and lysosomal biogenesis contributes to the neurodegeneration seen in SPG15 and related forms of HSP such as SPG11.

In 2012, Oz-Levi *et al.* identified a new form of apparently autosomal-recessive HSP, SPG49 (OMIM #615031), in three Jewish Bukharian families. Affected individuals present with distinct dysmorphic features, delayed development and muscular hypotonia at around 2 years of age before phenotypically progressing to intellectual disability, spastic paraplegia, rigidity, dysarthria and ataxia (Oz-Levi *et al.*, 2012). Severe central apnoea and gastrooesophageal reflux disease are additional features of the disease. Exome sequencing revealed a single and likely causal variant (c.3416delT) in the tectonin β-propeller containing protein 2 (*TECPR2)* gene *(*also *KIAA0329)*, resulting in a premature stop codon (Oz-Levi *et al.*, 2012). The truncated protein resulting from the putative pathogenic c.3416delT mutation undergoes rapid degradation, indicating a loss-of-function mechanism. Through high-throughput proteomic analysis of the autophagy pathway, TECPR2 has been established to be a binding partner of the mammalian Atg8 protein family, including LC3, and a probable positive regulator of autophagosome formation (Behrends *et al.*, 2010). Using fibroblasts of affected SPG49 patients and siRNA-mediated knockdown of TECPR2 in cultured cell lines, loss of TECPR2 was found to result in a decreased number of autophagosomes and reduced delivery of LC3 and p62 for lysosomal degradation. These results suggest that SPG49 pathology involves a significant, though not entirely complete, impairment of the autophagy pathway (Oz-Levi *et al.*, 2012). Providing insights into the mechanism of defective autophagy in SPG49, a recent study showed that TECPR2 is involved in maintaining functional ER exit sites, which may serve as scaffolds for the formation of autophagosomes (Stadel *et al.*, 2015).

AUTOPHAGY IN mTOR-ASSOCIATED NEURODEVELOPMENTAL DISEASES

Pathways that monitor nutrient or amino acid supply (mTORC1 pathway) and cellular ATP-levels (AMPK pathway) critically control autophagy. A central regulator of cellular metabolism, the ubiquitously expressed serine/threonine kinase mTORC1 facilitates anabolic processes that supply the basic building blocks for cell growth, differentiation and proliferation. Not surprisingly, mTORC1 also blocks catabolic pathways such as autophagy via transcriptional and post-translational mechanisms. Conversely, conditions known to induce autophagy, such as for example starvation or growth factor deprivation, effectively reduce mTORC1 activity. Critical targets of mTORC1 that mediate its effect on autophagy are TFEB, regulating autophagy at the transcriptional level, and the ULK1/2 complex, AMBRA1 and the ATG14L-associated VPS34 complex, proteins that are involved in the autophagy initiation step as discussed above (*Figure 2*). mTORC1 is important for disorders of autophagy for two reasons: firstly, genetic deficits of the mTOR pathway will almost certainly provoke changes in autophagy, and therefore "mTORpathies" can be regarded as "congenital disorders of autophagy regulation," and secondly and perhaps most importantly, mTORC1 inhibitors, including drugs currently approved for a variety of conditions, are clinically available inducers of autophagy. An example for an mTOR-associated neurodevelopmental disease with defective autophagy is tuberous sclerosis complex (TSC). In this multisystem disease, loss-of function mutations in *TSC1* or *TSC2* lead to a constitutive activation of the mTORC1 pathway (Lipton and Sahin, 2014, DiMario *et al.*, 2015). Overactive mTORC1 is the key pathogenic molecular mechanism in TSC and provides the scientific rationale for the use of mTORC1 inhibitors to treat this disease (Julich and Sahin, 2014, Ebrahimi-Fakhari and Sahin, 2015). Autophagy deficits in TSC have been implicated as contributors to epileptogenesis (McMahon *et al.*, 2012), brain malformations (Yasin *et al.*, 2013), tumour formation (Liang *et al.*, 2014), autism, and neurocognitive

deficits (Tang *et al.*, 2014) by impacting neuronal metabolism and synaptic signalling. Understanding autophagy dysfunction as a downstream event of *TSC1* or *TSC2* mutations is important and might lead to novel therapeutic targets. mTOR-associated diseases therefore highlight the close ties of the autophagy pathway to other fundamental signalling cascades and thus provide an opportunity to investigate the role of dysregulated autophagy in neurodevelopmental diseases.

AUTOPHAGY IN LYSOSOMAL STORAGE DISEASES

Progressive accumulation of undigested macromolecules in lysosomes is the hallmark feature of lysosomal storage diseases, a group of nearly 60 different diseases caused by mutations in genes encoding for lysosomal enzymes or membrane proteins (Boustany, 2013, Platt, 2014). Not surprisingly, impaired lysosomal metabolism has many effects on upstream pathways that mediate cargo delivery to the lysosomes. Recent studies have documented impaired autophagy in patient samples and disease models of many different lysosomal storage diseases (Lieberman *et al.*, 2012, Ebrahimi-Fakhari *et al.*, 2014). These deficits in autophagy are likely independent and significant contributors to neurodegeneration and other neuronal and non-neuronal disease manifestations, as convincingly illustrated in Niemann-Pick disease Type C (NP-C) models, where a block in autophagic flux and an accumulation of autophagosomes secondary to impaired maturation has been documented (Elrick *et al.*, 2012, Maetzel *et al.*, 2014, Sarkar *et al.*, 2014). Interestingly, re-expression of NPC1, the protein mutated in NPC, restored autophagy deficits in NPC1-deficient cells, and pharmacological stimulation of autophagy had a cell-protective effect. The latter concept was recently confirmed in iPSC-derived hepatic and neuronal cells from NPC patients, where mTOR-independent autophagy inducers, identified through a large-scale screening approach for small molecules, promoted cell viability (Maetzel *et al.*, 2014).

Combining compounds that mobilize lysosomal cholesterol, such as 2-hydroxypropyl-β-cyclodextrin (Vite *et al.*, 2015) with inducers of autophagy is thus a promising novel therapeutic approach. Glycogen storage disease type II or Pompe disease, although primarily a severe myopathy, is another important case for the potential therapeutic role of autophagy. Genetic approaches that employ TFEB to enhance autophagic flux have shown great promise in proof-of-principle *in vitro* and *in vivo* studies (Spampanato *et al.*, 2013). Future investigations for small molecules or effective genetic strategies that would render TFEB a clinically approachable target will prove or disprove the clinical utility of these findings for lysosomal storage diseases.

UNIFYING FEATURES IN CONGENITAL DISORDERS OF AUTOPHAGY

Major differences in the clinical manifestations of different congenital disorders of autophagy are obvious. This clinical heterogeneity may result from the fact that genes and proteins mutated in individual diseases map to different stages of the pathway and may lead to different degrees of residual autophagic activity. Clinical manifestations are also likely influenced by tissue-specific expression of autophagy proteins during and after development, differences in susceptibility of tissues to deficits in autophagy, a different degree of autophagic flux and metabolic activity in different cell types, and compensatory mechanisms or the existence of genetic or environmental modifiers. Identification and molecular delineation of novel congenital deficits of autophagy in humans will provide an understanding of these factors and will thus provide unique insights into the role of autophagy in human development and disease. Despite a variable clinical expression, a few unifying features are appreciable in congenital disorders of autophagy (*Box 1*). This includes, for example, a clinical manifestation in multiple organ systems with predominant

involvement of the nervous system, an onset in childhood or adolescence, a progressive disease course with neurodegenerative features, and a “storage phenotype” (*Box 1*). An involvement of the long white matter tracts as seen, for example, with agenesis or progressive thinning of the corpus callosum is another intriguing common denominator. How disruption of neuronal autophagy affects higher brain functions remains an open question and has implications for complex disease manifestations such as intellectual disability or epilepsy.

RESTORING DEFICITS IN AUTOPHAGY AS A THERAPEUTIC APPROACH

Pharmacological approaches that modulate autophagy have received increasing attention as a potential therapy for a broad range of autophagy-associated diseases (*Table 2*) (Ebrahimi-Fakhari *et al.*, 2012, Rubinsztein *et al.*, 2012, Nixon, 2013). Many small molecule inducers of autophagy act through the mTOR pathway (for example rapamycin and rapalogs), but mTOR-independent regulators also exist (such as for example carbamazepine or lithium which signal through inositol 1,4,5-trisphosphate; *Table 2*). Target specificity is a tremendous challenge for drug development, and many available compounds have autophagy-independent off-target effects. Not surprisingly, a number of known autophagy modulators are currently in clinical trials or in clinical use for indications other than autophagy induction. It is imperative to learn how these compounds affect autophagy and whether existing drugs with an extensive clinical safety profile could be readily employed to target autophagy. This concerns, for example, the use of the antiepileptic drug carbamazepine (Hidvegi *et al.*, 2010, Maetzel *et al.*, 2014) or lysosomotropic agents such as chloroquine (Amaravadi *et al.*, 2011). Although the diseases discussed in this review are rare, the extent to which they might inform us about therapeutic target in the autophagy pathway is significant. Based on current evidence, enhancing residual autophagy function or bypassing molecular deficits

appear to be rational therapeutic approaches for congenital disorders of autophagy. The latter might be achieved by targeting the stage of the autophagy pathway that is specifically disrupted in each given disease. Understanding the biology of congenital disorders of autophagy will help to design these proposed stage-specific autophagy-targeted therapies.

Induced pluripotent stem cells (iPSCs) have emerged as a valuable pre-clinical model to study rare diseases, given that reliable animal models for these conditions are often not readily available. In addition, several protocols exist that enable differentiation into relevant cell types such as neuronal cells. These will be instrumental for understanding disease phenotypes arising from different genetic mutations in individual patients and will provide a very useful platform for high throughput screening approaches that aim to identify molecules that restore autophagy function in a given disease. Recent studies have described the generation of human iPSC lines from patients with a range of inherited diseases (Park *et al.*, 2008, Ebert *et al.*, 2009, Lee *et al.*, 2009, Marchetto *et al.*, 2010) and proof-of-principle screening studies for modulators of autophagy using iPSC are emerging (Maetzel *et al.*, 2014).

The transition from cellular and mouse models to humans will contest the potential for harnessing autophagy-based therapies. To translate potential targets into therapies, a challenge that will have to be overcome is the development of *in vivo* biomarkers to detect changes in autophagy in response to therapeutics or disease progression. This will be most effective when stage-specific methods or surrogate biomarkers become available to confirm target engagement, which will be essential to interpret outcomes in clinical trials. As with most congenital childhood-onset diseases with a clear neurodevelopmental phenotype, another concern is the optimal timing to start treatment. Along the same lines, it remains to be seen

whether existing lesions, such as for example iron storage in the basal ganglia of BPAN patients, are reversible by correcting autophagy deficits.

CONCLUSIONS

Genetic disruption of the autophagy-lysosomal pathway, a fundamental metabolic pathway, results in a new and diverse group of multisystem diseases. We therefore argue that based on their shared genetic and molecular characteristics, these should be collectively termed *congenital disorders of autophagy*, a novel subclass within the field of inborn errors of metabolism. In the past two years alone, next-generation sequencing technologies have allowed for the identification of a number of pathogenic mutations in core autophagy genes and will likely continue to reveal novel single gene disorders of this crucial cellular pathway. Bridging the gap between the identification of causative genes to the delineation of molecular disease mechanisms is an upcoming challenge. Development of disease models, such as those generated through iPSC technology, will allow us to gain insights into potential therapeutic targets and will yield novel therapies. Insights into congenital diseases of autophagy may be important to a wide spectrum of more common sporadic diseases with defective autophagy.

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CONFLICT OF INTEREST

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FIGURE LEGENDS

Figure 1. The Three Main Subtypes Of Autophagy And Their Implications For Multiple Tissues And Organ Systems

Autophagy mainly comprises of three subtypes: microautophagy, chaperone-mediated autophagy and macroautophagy. These are distinguished based on the route and mechanism of cargo delivery to lysosomes, the final degrading organelles. Research over the last decades has elucidated a number of cell and tissue-specific functions that depend on or are critically influenced by autophagy. These functions are present in many organ systems, including the nervous system. The variety of functions of autophagy in different organ systems under physiological conditions is emphasized by the broad clinical manifestations in congenital disorders of autophagy, which manifest as multisystem diseases with prominent CNS pathology. Abbreviations: CMA (chaperone-mediated autophagy).

Figure 2. Macroautophagy – An Overview Of The Molecular Pathway And Mutations Associated With Congenital Disorders Of Autophagy

Macroautophagy is a step-wise process resulting in the formation of double-membrane-bound autophagic vesicles that engulf their cargo before fusing with lysosomes. The principle stages of macroautophagy include: (I) initiation, (II) nucleation of an isolation membrane (also called phagophore), (III) elongation of evolving autophagic vesicles, (IV) engulfment of cargo and closure of the autophagosomal membrane, (V) autophagosome maturation, (VI) fusion with late endosomes or lysosomes, and finally (VII) degradation of cargo through lysosomal hydrolases. The last step yields basic metabolites that are then recycled. Mutations in congenital disorders of autophagy and single gene disorders associated with deficits in the regulation of autophagy impair different stages of the pathway.

Through interfering with the Beclin1 complex, hereditary spastic paraplegia-associated recessive mutations in *ZFYVE26* (SPG15) impair early stages such as the formation of the isolation membrane. Mutations in *TECPR2* (SPG49) were recently shown to be critically involved in maintaining endoplasmic reticulum exit sites that may serve as scaffolds for the formation of early autophagosome intermediates. X-linked *WDR45* mutations cause beta-propeller protein-associated neurodegeneration (BPAN) and have been found to potentially interfere with the elongation of nascent autophagic vesicles. Autosomal-recessive *EPG5* mutations in Vici syndrome as well as *SNX14* mutations in *SNX14*-associated autosomal-recessive cerebellar ataxia and intellectual disability syndrome impact the late stages of the autophagy pathway through impairing autophagosome-lysosome fusion. Mutations in *SPG11* (SPG11) lead to a defect in autophagic lysosome reformation. Autophagy-associated diseases also affect different stages of autophagy regulation. An example for mTOR-associated neurodevelopmental diseases, loss of function mutations in *TSC1* or *TSC2* in tuberous sclerosis complex lead to constitutive activation of mTORC1 and thus block autophagic flux at multiple stages. Locating the defect to the late stages of the pathway, lysosomal storage diseases impact lysosomal metabolism and thus block upstream steps in the autophagy pathway. Impaired crosstalk between the lysosomal pathway and autophagosome biogenesis might also impact the coordinated regulation of both compartments. Abbreviations: AR-CAID (autosomal-recessive ataxia and intellectual disability syndrome); BPAN (beta-propeller protein-associated neurodegeneration); HSP (hereditary spastic paraplegia); LSD (lysosomal storage disease); TSC (tuberous sclerosis complex).

Figure 3. Vici Syndrome – A Multisystem Disease

Mutations in the autophagy gene *EPG5* cause Vici syndrome, a paradigm for multisystem diseases associated with defective autophagy. The eight cardinal features of Vici syndrome consist of agenesis of the corpus callosum, acquired microcephaly, bilateral cataracts, hypertrophic or dilated cardiomyopathy, combined immunodeficiency, and skin, hair or retinal hypopigmentation, failure to thrive and profound developmental delay. CNS manifestations are manifold and include congenital brain malformations such as agenesis of the corpus callosum with colpocephaly, cerebellar hypoplasia, hypoplasia/atrophy of the brain stem, abnormalities of the septum pellucidum, opercular hypoplasia and probably age-dependent abnormal T2 signal in the thalami. Dysgenesis of the falx, non-lissencephalic cortical dysplasia, polymicrogyria of the cerebral hemispheres, or bilateral schizencephaly have also been reported in a few individuals. Delayed myelination and diffuse white matter atrophy are commonly reported. Pointing to the potential presence of a neurodegenerative phenotype in addition to prominent deficits in brain development, dilated ventricles, Purkinje cell loss, and diffuse cerebral atrophy have been described in a subset of patients.

Figure 4. Beta-Propeller Protein-Associated Neurodegeneration (BPAN) – Natural History Of A Biphasic Disease

Patients with *WDR45*-mutation associated BPAN commonly present with a distinct biphasic clinical course. Initial manifestations in infancy or early childhood consist of global developmental delay, intellectual disability, and seizures, which tend to progress in the later stages of the disease. Cerebellar ataxia, spasticity, Rett-like hand stereotypies, and autistic features have also been reported in a subset of patients. The disease advances in adolescence or early adulthood when dystonia, Parkinsonism, and progressive cognitive decline become prominent. Other

manifestations often include bladder and bowel incontinence, disordered sleep, and visual and auditory deficits.

Table 1. Congenital disorders of autophagy			
Disease (# OMIM)	**Gene**	**Affected stage of the autophagy pathway**	**Neurological Manifestations**
Vici syndrome (#242840)	*EPG5*	Late stages: • Autophagosome-lysosome fusion • Proteolysis within autolysosomes	• Agenesis of the corpus callosum and other congenital brain malformations • Delayed myelination and white matter atrophy • Acquired microcephaly and dysmorphic features • Developmental delay / developmental regression • Failure to thrive • Muscular hypotonia • Seizures • Sensorineural deafness
Beta-propeller protein-associated neurodegeneration (#300894)	*WDR45*	Early stages: • Autophagosome elongation	*Childhood:* • Developmental delay / intellectual disability • Seizures • Spastic paraplegia • Rett-like stereotypies • Autistic features *Adolescence:* • Progressive dementia • Parkinsonism, dystonia • Optic nerve atrophy • Sensorineural hearing loss • Bladder and bowel incontinence • Sleep disorder
SNX14-associated autosomal-recessive cerebellar ataxia	*SNX14*	Late stage: • Autophagosome-lysosome fusion (probably)	• Progressive cerebellar atrophy and ataxia • Developmental delay / intellectual disability

and intellectual disability syndrome (#616105)			• Muscular hypotonia • Seizures • Autistic features • Storage disease phenotype
Hereditary spastic paraplegia - SPG11 (#604360) & Hereditary spastic paraplegia - SPG15 (#270700)	*SPG11 & ZFYVE 26*	SPG11: Late stage: • Lysosome reformation / biogenesis SPG15: Early, middle and late stages: • Autophagosome initiation / nucleation • Autophagosome maturation • Autophagosome-lysosome fusion • Lysosome reformation / biogenesis	• Progressive spasticity and weakness of the lower limbs • Developmental delay / cognitive decline • Thinning of the corpus callosum • Axonal, motor, or sensorimotor peripheral neuropathy • Pseudobulbar involvement with dysarthria and dysphagia *Less common:* • Retinal degeneration (Kjellin syndrome) • Cerebellar ataxia • Parkinsonism
Hereditary spastic paraplegia - SPG49 (#615031)	*TECPR 2*	Early stages: • Autophagosome nucleation (probably)	• Progressive spasticity and weakness of the lower limbs • Developmental delay / cognitive decline • Muscular hypotonia • Microcephaly and dysmorphic features • Cerebellar dysfunction • Central apnoea • Seizures • Thinning of the corpus callosum

Table 2. Modulators Of Autophagy In Neuronal Disease Models		
Compound	**Proposed Target and Mechanism**	**Model System & Selected References**
Bafilomycin A1	Autophagy Inhibition • Inhibition of the vacuolar-type ATPase • Disrupts lysosomal acidification and prevents autophagosome-lysosome fusion	Neuronal cell models (Shacka *et al.*, 2006, Bains *et al.*, 2009); mouse models (Ebrahimi-Fakhari *et al.*, 2011, Klucken *et al.*, 2012)
Carbamazepine	Autophagy induction • Voltage-gated Na^{2+} channel inhibitor • Lowers inositol and inositol-1,4,5-triphosphate levels	Neuronal cell models (Williams *et al.*, 2008, Xiong *et al.*, 2011); iPSC-derived neuronal cells (Maetzel *et al.*, 2014); mouse models (Wang *et al.*, 2012a, Li *et al.*, 2013)
(Hydroxy-) Chloroquine	Autophagy inhibition • Disrupts lysosomal acidification and prevents autophagosome-lysosome fusion	Neuronal cell models (Boland *et al.*, 2010); mouse models (Ebrahimi-Fakhari *et al.*, 2011, Vodicka *et al.*, 2014)
Clonidine	Autophagy induction • Imidazole receptor agonist • Lowers cAMP levels	Neuronal cell model; *Drosophila melanogaster*; zebrafish (Williams *et al.*, 2008)
Latrepirdine	Autophagy induction • mTORC1 inhibition	Neuronal cell model; mouse models (Steele *et al.*, 2013a, Steele *et al.*, 2013b)
Lithium	Autophagy induction • Inositol monophosphatase inhibitor • Lowers inositol and inositol-1,4,5-triphosphate levels	Neuronal cell models (Sarkar *et al.*, 2005, Chang *et al.*, 2011); mouse models (Sarkar *et al.*, 2005, Fornai *et al.*, 2008, Shimada *et al.*, 2012, Duarte-Silva *et al.*, 2014)
Nilotinib	Autophagy induction • Abl tyrosine kinase inhibitor • Activates AMPK	Mouse models (Hebron *et al.*, 2013, Lonskaya *et al.*, 2014)

Rapamycin and rapalogs	Autophagy induction • mTORC1 inhibition	Neuronal cell models (Webb *et al.*, 2003, Pan *et al.*, 2008b, Williams *et al.*, 2008); mouse models (Ravikumar *et al.*, 2004, Menzies *et al.*, 2010, Majumder *et al.*, 2011, Cortes *et al.*, 2012, Viscomi *et al.*, 2012, Jiang *et al.*, 2014)
Resveratrol	Autophagy induction • Sirtuin-1 activation • Activates AMPK	Neuronal cell model (Vingtdeux *et al.*, 2010); mouse model (Vingtdeux *et al.*, 2010, Shu *et al.*, 2015)
Rilmenidine	Autophagy induction • Imidazole receptor agonist • Lowers cAMP levels	Neuronal cell model (Williams *et al.*, 2008, Rose *et al.*, 2010); mouse model (Rose *et al.*, 2010)
SMER28 and other SMER	Autophagy induction • Unknown mechanism, likely mTOR-independent	Neuronal cell model (Tian *et al.*, 2011); *Drosophila melanogaster* (Sarkar *et al.*, 2007b)
Trehalose	Autophagy induction • Chemical chaperone	Neuronal cell model (Sarkar *et al.*, 2007a); mouse models (Rodriguez-Navarro *et al.*, 2010, Schaeffer *et al.*, 2012, Castillo *et al.*, 2013, Zhang *et al.*, 2014, He *et al.*, 2015, Li *et al.*, 2015)
Valproate	Autophagy induction • Myo-inositol-3-phosphate synthase inhibitor • Lowers inositol and inositol-1,4,5-triphosphate levels	Neuronal cell model; *Drosophila melanogaster* (Williams *et al.*, 2008)
Verapamil (and other Ca^{2+}-channel blockers)	Autophagy induction • L-type Ca^{2+}-channel antagonist • Lowers intracellular calcium levels	Neuronal cell model; *Drosophila melanogaster*; zebrafish (Williams *et al.*, 2008)

Box 1. Unifying clinical characteristics of congenital disorders of autophagy

- Disease onset in early childhood / adolescence
- Prominent nervous system involvement
    - Multiple brain regions involved
    - Long white-matter tracts (corpus callosum and cortico-spinal tract) and the cerebellum (Purkinje cells) are often affected
    - Neurodegenerative phenotype with developmental regression and cognitive decline
    - Storage disease phenotype
    - Delayed development / intellectual disability, muscular hypotonia, seizures and movement disorders are common
- Often multisystem disease with additional non-neuronal manifestations (including myopathy and ophthalmic manifestations)
- Progressive disease course, often fatal